Research Article

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Natural-product-based chromenes as a novel class of potential termiticides

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Abstract

BACKGROUND: Among the termite infestations in the United States, the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), is considered to be the most devastating termite pest. This pest most likely invaded North America as a result of the disembarkation of wooden military cargo at the port of New Orleans that arrived from Asia during and after World War II. It has now spread over other states, including Texas, Florida, South Carolina and California. Devastation caused by *C. formosanus* in North America has been estimated to cost $US 1 billion a year. Over the past decades, organochlorines and organophosphates, the two prominent classes of termite control agents, have been banned owing to environmental and human health concerns. At the present time, phenylpyrazoles, pyrethroids, chloronicotinyls and pyrroles are being used as termite control agents. Mammalian toxicity and seeping of these compounds into groundwater are some of the drawbacks associated with these treatments. The instruction for the application of these termiticides indicate ground water advisory. Hence, with the increasing spread of termite infestation there is an increased need to discover effective, environmentally friendly and safe termite control agents with minimal mammalian toxicity.

RESULTS: Chromene analogs derived from a natural-product-based chromene amide isolated from *Amyris texana* were tested in a collaborative discovery program for effective, environmentally friendly termite control agents. Several chromene derivatives were synthesized and characterized as a novel class of potential termiticides, followed by bioassays. These compounds exhibited significantly higher mortalities compared with untreated controls in laboratory bioassays.

CONCLUSION: Chromene derivatives have been shown to be a potential novel class of termiticides against Formosan subterranean termites.

Keywords: Formosan subterranean termites; *Amyris Texana*; chromene amides; chromenes; termiticide

1 INTRODUCTION

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), most likely invaded North America as a result of disembarkation of wooden military cargo at the port of New Orleans that arrived from Asia during and after World War II.[1,2,3] It is native to East Asia and is among the most devastating termite pests. It is believed that introduction of this pest in Hawaii occurred in a much earlier period owing to sandalwood trade from the Far East. New Orleans and the surrounding area have the highest termite population in the US mainland, and it causes heavy damage to the historic French Quarter, households and even non-cellulosic materials such as underground cable systems in the surrounding areas.[4,5] Over 50 species of trees, including New Orleans' majestic live oaks, are infested by this pest.[6–8] During the past 50 years since the first invasion of this pest in North America, in the New Orleans area it has flourished rapidly in the warm, humid climate.[4] It has now spread over other states, including Texas, Florida, South Carolina, Alabama, Tennessee, Georgia and California.[3] Devastation caused by *C. formosanus* in North America has been estimated to cost $US 1 billion a year.[4,9] In New Orleans alone it is estimated to cost $US 300 million annually. Owing to the recent hurricanes that affected the southern states, particularly Louisiana, there is a possibility that infestation of termites may increase owing to a tremendous amount of wood and other celluloid debris buried in the soil, which provide an ideal breeding environment. Property owners in the heavily infested areas are frustrated with the pest control professionals because of their inability to control this pest.[3] Over the past two decades, organochlorines and organophosphates, the two prominent classes of termite control agents, have been banned owing to environmental and human health concerns.[10] At the present time, phenylpyrazoles, pyrethroids, chloronicotinyls and pyrroles are being used as termite control agents. Mammalian toxicity and the seeping of these compounds into groundwater are some of the drawbacks associated with these treatments.[11] Hence, with the increasing spread of termite infestation there is an

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increased need to discover effective, environmentally friendly and safe termite control agents with a minimal mammalian toxicity.

As part of USDA efforts to search for effective, environmentally friendly termite control agents, the authors have screened some natural products isolated from plant extracts belonging to various families. They have postulated that secondary metabolites produced by plants as part of their natural defense mechanism represent one area of exploration in the search for such termite control agents. Secondary metabolites play a significant role in defending plants from insects, fungi, bacteria and other plants. This paper describes the isolation and identification of a lead molecule and the synthesis and structure activity of a series of derivatives based on the natural product lead from *Amyris Texana*.

2 MATERIALS AND METHODS

2.1 General experimental procedures

Extracts were analyzed on silica gel GF TLC plates with a fluorescent indicator (250 µm; Analtech, Newark, DE). Iodine vapor, UV light (at 254 and 365 nm) and Dragendorff and anisaldehyde spray reagents were used for the detection of compounds. Column chromatography was carried out with kieselgel 60 (particle size 0.063–0.2 mm; Merck) with mixtures of hexane and acetone in varying amounts. All solvents were reagent grade and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded either on a Bruker AMX NMR spectrometer operating at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR or on a Varian Mercury AS400 spectrometer operating at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR. The HR-ESIMS was measured using a Bruker QTOF micromass spectrometer or using a Jeol ACCU TOF JMS-T1000 mass spectrometer. GC-MS analysis was carried out on an HP5790 MSD spectrometer (Hewlett Packard, USA) equipped with GC 5890 using a DB-1 column (20 m × 0.2 mm, 0.18 µm film thickness). The oven was temperature programmed from 60 °C (5 min) to 280 °C (20 min) at 5 °C/min, with helium as the carrier gas.

2.2 Plant material

Leaves of *A. texana* were collected in Cameron County in South Texas in June 2002 by Dr Charles Burandt at the University of Mississippi. A voucher specimen (BUR 190 204 a) is deposited at the University of Mississippi herbarium. The leaves were air dried, ground and stored at room temperature until they were extracted.

2.3 Extraction and fractionation

Air-dried and ground *A. texana* leaves (500 g) were extracted repeatedly at room temperature (25–28 °C) with ethyl acetate (2 L × 3) by stirring with a magnetic stirrer. The resulting extracts were filtered through filter paper (Whatman No. 1) and combined, and the solvent was evaporated at 40 °C under reduced pressure to afford a dark-green residue (21.6 g). This residue (20.0 g) was subjected to column chromatography on silica gel eluting with hexane, and then with increasing amounts of acetone (up to 100%). Fractions of 400 mL were collected, and the elution profile of the column was monitored by TLC plates sprayed with anisaldehyde, Dragendroff spray reagents and I_2 vapor. The fractions with similar TLC profiles were combined to yield 34 fractions.

N-[2-(2,2-Dimethyl-2H-chromen-6-yl)-ethyl]-3,*N*-dimethyl-butyramide, chromene amide (**1**): the pale-yellow oily mass in fraction 23 was further purified by silica column chromatography with 10% EtOAc in hexane to yield an oil (620 mg, 0.12%) and characterized by spectroscopic methods according to a published method.[12–14]

2.4 Syntheses of analogs

Compounds **2** to **14** (Fig. 3) were prepared and identified using spectroscopic data according to published methods.[12–15] The general synthetic procedure involved reaction of the appropriate phenol with 3-chloro-3-methyl-1-butyne, followed by pyran ring formation by heating with *N*,*N* diethylaniline (Fig. 2).

1-[4-(1,1-Dimethyl-prop-2-ynyloxy)-2-hydroxy-phenyl]-ethanone (**15**): 2′–4′-dihydroxyacetophenone (25 mmol, 3.12 g) was refluxed under N_2 with a mixture of KI (6 g), anhydrous K_2CO_3 (6 g) and 3-chloro-3-methyl-1-butyne (6.8 mL, 60.5 mmol) in acetone (30 mL) for 48 h. The mixture was allowed to cool to room temperature and was then filtered, and the residue was washed with acetone. The combined acetone solution was evaporated to afford a gum. The major compound of this gum was isolated by silica gel flash column chromatography with 5% ether in hexane to afford compound **15** (1.1 g). ^{1}H NMR ($CDCl_3$) δ: 1.7 (6H, s), 2.5 (3H, s), 2.7 (1H, s), 6.6 (1H, d, J = 8 Hz), 6.8 (1H, s), 7.6 (1H, d, J = 8 Hz), 12.6 (1H, s).

1-(5-Hydroxy-2,2-dimethyl-2H-chromen-6-yl)-ethanone (**16**): compound **15** (1.0 g, 4.5 mmol) and *N*,*N*,-diethylaniline (10 mL) were refluxed under N_2 with stirring (210–220 °C) for 1 h. The reaction mixture was allowed to cool to room temperature and then diluted with diethyl ether (300 mL). The ether solution was washed with 6 M aqueous HCl (100 mL × 2) followed by saturated aqueous NaCl, and dried over anhydrous Na_2SO_4. Ether was removed under reduced pressure to afford a pale-yellow oil which was purified by silica gel column chromatography using 5% acetone in hexane to afford compound **16** as the major compound (yield 853 mg, 3.9 mmol, 86%). ^{1}H NMR ($CDCl_3$) δ: 1.41 (6H, s), 2.49 (3H, s), 5.53 (1H, d, J = 10 Hz), 6.28 (1H, d, J = 9 Hz), 6.67 (1H, d, J = 10 Hz), 7.45 (1H, d, J = 9 Hz), 12.95 (1H, s).

2-(1,1-Dimethyl-prop-2-ynyloxy)-4-methoxy-benzoic acid methyl ester (**17**): 2-hydroxy-4-methoxybenzoic acid methyl ester (4.15 g, 25 mmol) was refluxed under N_2 with a mixture of KI (6 g), anhydrous K_2CO_3 (6 g) and 3-chloro-3-methyl-1-butyne (6.8 mL, 60.5 mmol) in acetone (30 mL) for 48 h. The mixture was allowed to cool to room temperature and then filtered, and the residue was washed with acetone. The combined acetone solution was evaporated to afford a yellow gum which was dissolved in diethyl ether (200 mL) and partitioned between 1 M aqueous NaOH (300 mL × 2). The ether layer was dried over anhydrous $MgSO_4$ and evaporated to afford a pale-yellow oil. The major compound of this oil was isolated by silica gel flash column chromatography with 5% ether in hexane to afford compound **17** (yield 3.1 g, 12.5 mm, 50%). ^{1}H NMR ($CDCl_3$) δ: 1.37 (6H, s), 2.46 (3H, s), 2.59 (1H, s), 3.76 (3H, s), 5.47 (1H, d, J = 10 Hz), 6.22 (1H, d, J = 9.6 Hz), 6.36 (1H, s).

5-Methoxy-2,2-dimethyl-2H-chromene-8-carboxylic acid methyl ester (**18**): compound **17** (800 mg, 3.22 mmol) and *N*,*N*-diethylaniline (10 mL) were refluxed under N_2 with stirring (210–220 °C) for 1 h. The reaction mixture was allowed to cool to room temperature and then diluted with diethyl ether (300 mL). The ether solution was washed with 6 M aqueous HCl (100 mL × 2) followed by saturated aqueous NaCl, and dried over anhydrous Na_2SO_4. Ether was removed under reduced pressure to afford a pale-yellow oil which was purified by silica gel column chromatography with 5% acetone in hexane to afford compound **18** as pale-yellow crystals (yield 609 mg, 2.4 mmol, 74%). ^{1}H NMR ($CDCl_3$) δ: 1.37 (6H, s), 2.47 (3H, s), 3.72 (3H, s), 5.47 (1H, d, J = 10 Hz), 6.22 (1H, d, J = 9.6 Hz), 6.36 (1H, d, J = 8.4 Hz), 6.87 (1H, d,

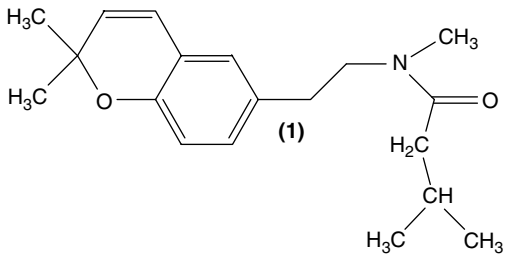

Figure 1. Structure of chromeme amide (**1**).

J = 8 Hz); ^{13}C NMR ($CDCl_3$) δ: 27.87, 22.34, 55.74, 76.88, 103.13, 115.19, 121.05, 127.33, 128.60, 150.27, 156.51, 201.67.

6,6-Dimethyl-6H-[1,3]dioxolo[4,5-g]chromene (**19**): 4-hydroxy-1,2-methylenedioxybenzene (3.4 g, 25 mmol) was refluxed under N_2 with a mixture of KI (6 g), anhydrous K_2CO_3 (6 g) and 3-chloro-3-methyl-1-butyne (6.8 mL, 60.5 mmol) in acetone (30 mL) for 48 h. The mixture was allowed to cool to room temperature and then filtered, and the residue was washed with acetone. The combined acetone solution was evaporated to afford a brownish-yellow gum which was dissolved in diethyl ether (200 mL) and partitioned between 1 M aqueous NaOH (300 mL × 2). The ether layer was dried over anhydrous $MgSO_4$ and evaporated to afford a pale-yellow oil. Compound **19** was isolated by silica gel flash column chromatography with 5% ethyl acetate in hexane (yield 2.4 g, 11 mm, 44%). ^{1}H NMR ($CDCl_3$) δ: 1.40 (6H, s), 5.45 (1H, d, J = 10 Hz), 5.85 (2H, s), 6.18 (1H, d, J = 10 Hz), 6.41 (1H, s), 6.47 (1H, s). HRMS (ESI-TOF) m/z 205.086376 $[M + H]^+$ (calcd for $C_{12}H_{22}O_3$, 205.086470).

2.5 Bioassay for termiticide activity

Termites from four colonies of *C. formosanus* were obtained from field sites in New Orleans, Louisiana, from bucket traps[16] and maintained on spruce (*Picea* spp.) slats (10 × 4 × 0.5 cm) under conditions of ca 100% relative humidity and 26.6 °C. Termites were identified using keys for soldier identification from Scheffrahn and Su.[17]

The test solutions for the assays were prepared by dissolving the pure compounds in acetone. A quantity of 100 µL of acetone solution was pipetted onto 2.5 cm diameter Whatman No. 1 filter paper, resulting in a treatment with a specific weight percentage of active ingredient or extract to weight of filter paper. Acetone was allowed to evaporate from the filter paper for several hours until it was dry. Treated filter paper disks were placed in plastic petri dishes (35 × 10 mm) and moistened with 100 µL water. Twenty *C. formosanus* workers (third instar or greater, as determined by size) and two soldiers were placed on each treatment. Treatments were replicated 4 times with termites for each replicate originating from a different *C. formosanus* colony. Petri dishes were covered and maintained at ca 100% RH and 26.6 °C. Filter paper disks receiving water alone served as controls in the experiment. It was previously determined that the filter paper treated with acetone alone had no discernible effect on termite mortality or consumption.

2.6 Statistical analysis

Daily termite mortality was evaluated for 21 days. Cumulative daily mortality (mean and standard deviation) was calculated for each treatment. Treatments from the same experiment (grouped by table) were compared using ANOVA (PROC GLM; SAS Institute, 1990), and means were separated with the Student–Newman–Keuls means separation test (SNK), following transformation to arcsine square root percentage mortality ($P < 0.05$; PROC GLM, SAS Institute, 1990).[18] Actual percentage mortality is reported in the tables 1 and 2.

3 RESULTS AND DISCUSSION

Chromene amide (**1**) was isolated from the ethyl acetate extract of the leaves of *A. texana* (Fig. 1). A preliminary termiticide bioassay of this compound indicated the presence of moderate activity (~35% mortality). Based on this finding, a series of chromene derivatives were synthesized as shown in Fig. 3 and tested for termiticide activity (Table 1). At 1% (wt/wt), analogs **6, 12, 19** and **13** are fast acting, with 90–100% mortality at 4 days after treatment (DAT). Analogs **2, 7** and **11** acted somewhat more slowly, showing 100% mortality at 11 DAT at 1% (wt/wt). Analog **9** with an aldehyde group showed high activity, with 81% mortality at 8 DAT up to 98% mortality at 15 DAT. Analogs **3, 4, 8, 10** and **5** showed a range of activities from 2.5 to 88% mortality (Table 1). Compounds **16, 18** and **19** also showed high mortality rates (85–100%) when tested at 2% (wt/wt) (Table 2). Compounds **16** and **18** showed 100% mortality at 15 and 17 DAT, whereas compound **19** showed 85% mortality at 21 DAT.

These analogs were further evaluated in a dose-dependent manner with concentrations ranging from 0.05 to 0.001 wt/wt Analogs **13, 16** and **18** showed the highest activity, with 100% mortality at 5 DAT at 0.5% (wt/wt). Analog **7**, which is a regioisomer of **18**, where the CH_2OH is at C-7, was not as active as analog **13**. Therefore, the CH_2OH group at C-5 plays an important role in the activity of these chromene analogs. Substitution of methyl ester groups at these positions (**11** and **12**) did not enhance the activity. According to the dose-dependent mortality data (data not shown), analogs **13, 16** and **18** showed 100% mortality at 0.5% (wt/wt). At concentrations below 0.5%, these analogs were less effective.

In the literature there are reports of chromene analogs as insecticides,[19–21] but this is the first report of chromene analogs as termiticides. Some chromene derivatives possess juvenile hormone antagonistic activities.[22,23] Precocenes are plant-derived

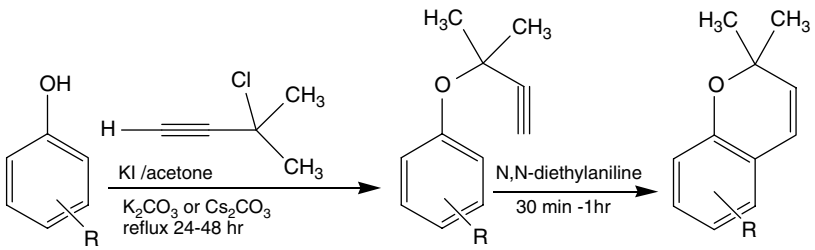

Figure 2. General procedure for synthesis of chromene analogs.

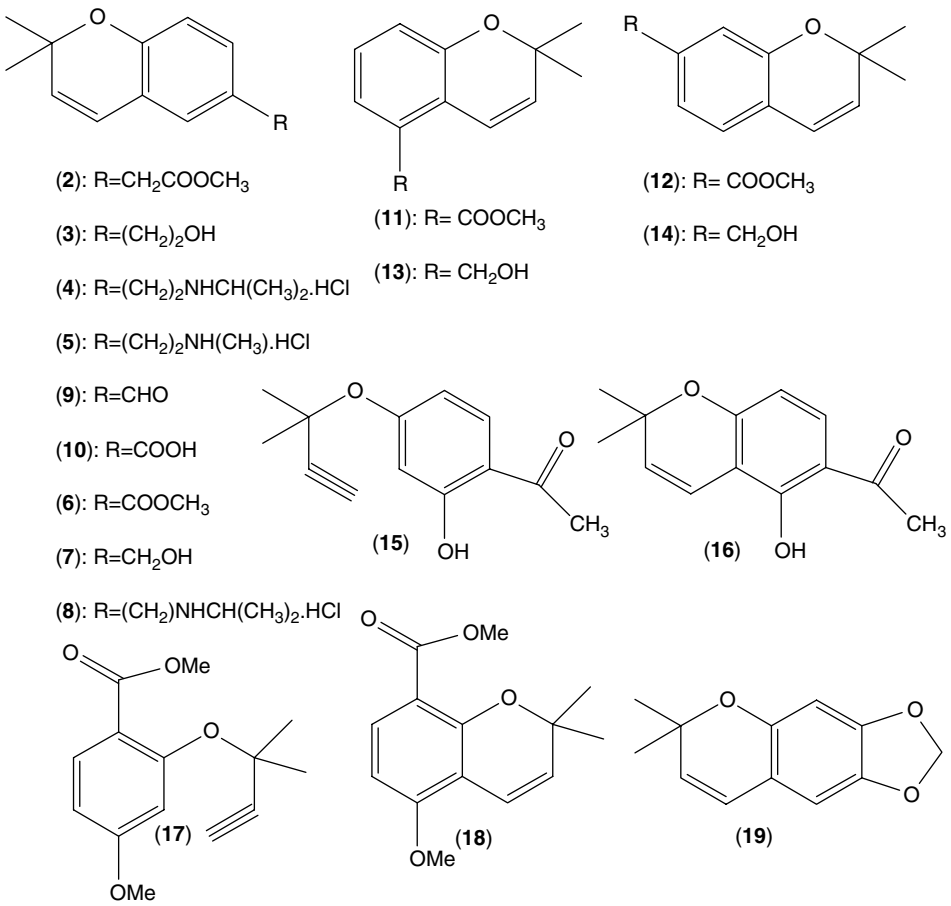

Figure 3. Structures of chromene analogs.

Table 1. Cumulative percentage mortality of *C. formosanus* on filter paper[a]

	% Mortality (mean ± SD)						
	Days						
Compound 1% (wt/wt)	1	4	8	11	15	18	21
Chromene amide **1**	6.3 ± 12.5 C	32.5 ± 45.6 CD	32.5 ± 45.6 B	32.5 ± 45.6 C	32.5 ± 45.6 C	32.5 ± 45.6 C	32.5 ± 45.6 B
2	12.5 ± 6.5 BC	82.5 ± 20.6 AB	92.5 ± 15.0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A
3	0 C	10.0 ± 10.8 DE	33.8 ± 41.7 B	56.3 ± 32.0 B	70.0 ± 31.9 B	71.3 ± 30.7 B	73.8 ± 30.4 A
4	3.75 ± 7.5 C	3.8 ± 7.5 E	3.8 ± 7.5 C	5.0 ± 10.0 D	7.5 ± 8.7 D	8.8 ± 7.5 D	15.0 ± 7.1 BC
5	0 C	0 E	0 C	1.3 ± 2.5 D	2.5 ± 2.9 D	2.5 ± 8.9 D	3.8 ± 4.8 C
6	3.75 ± 7.5 C	92.5 ± 15.0 AB	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A
7	1.3 ± 2.5 C	40.0 ± 35.1 CD	92.5 ± 11.9 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A
8	8.8 ± 17.5 C	20.0 ± 14.7 DE	55.0 ± 30.3 B	72.5 ± 23.3 B	80.0 ± 21.2 AB	88.8 ± 13.1 AB	88.8 ± 13.1 A
9	51.3 ± 42.7 A	62.5 ± 40.9 BC	81.3 ± 22.5 A	93.8 ± 7.5 A	98.8 ± 2.5 A	98.8 ± 2.5 A	98.8 ± 2.5 A
10	0 C	0 E	0 C	0 D	1.3 ± 2.5 D	1.3 ± 2.5 D	2.5 ± 5.0 C
11	0 C	87.5 ± 18.9 AB	97.5 ± 2.9 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A
12	32.5 ± 29.9 ABC	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A
13	41.3 ± 40.5 AB	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A
14	26.3 ± 16.5 ABC	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A	100.0 ± 0 A
Untreated	0 C	0 E	1.3 ± 2.5 C	1.3 ± 2.5 D	1.3 ± 2.5 D	1.3 ± 2.5 D	2.5 ± 5.0 C

[a] Twenty workers (≥ third instar)/one soldier per rep., three reps, three colonies.
Means within a column/treatment with the same letter are not significantly different, SNK: $P < 0.05$.
Twenty workers (≥ third instar)/two soldiers per rep., four reps, four colonies.

Table 2. Cumulative percentage mortality of *C. formosanus* on filter paper[a]

Compound 1% (wt/wt)	% Mortality (mean ± SD)						
	Days						
	1	3	7	9	15	17	21
16	0 A	1.3 ± 2.5 A	66.3 ± 31.2 A	98.8 ± 2.5 A	100 ± 0 A	100 ± 0 A	100 ± 0 A
18	0 A	2.5 ± 5.0 A	30.0 ± 10.8 B	61.3 ± 10.3 B	96.3 ± 4.8 A	100 ± 0 A	100 ± 0 A
19	0 A	2.5 ± 5.0 A	12.5 ± 6.5 BC	23.8 ± 14.9 C	75.0 ± 25.2 B	82.5 ± 12.6 B	85.0 ± 12.2 B
Untreated	0 A	0 A	3.8 ± 4.8 C	3.8 ± 4.8 D	5.0 ± 4.1 C	5.0 ± 4.1 C	5.0 ± 4.1 C

[a] Twenty workers (≥ third instar)/two soldiers per rep., four reps, four colonies.
Means within a column/treatment with the same letter are not significantly different, SNK: $P < 0.05$.

chromenes with insecticidal activities. In the present authors' lab, the antifungal and algicidal activities of some chromene derivatives have also been demonstrated.[14] *Amyris Texana* belongs to the plant family Rutaceae, which is known to have various biologically active secondary metabolites with antimicrobial and insecticidal activities. Thus, the presence of chromene amide (**1**) in the leaves of *Amyris texana* may be of ecological importance for the survival of the plant in the ecosystem. The chromene analogs synthesized on the basis of **1** have the potential to be further developed as insecticides.

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